Investigations of La Rioja Terroir for Wine Production Using ¹H NMR Metabolomics

Eva López-Rituerto,[†,§] Francesco Savorani,[‡,§] Alberto Avenoza,[†] Jesús H. Busto,*[,†] Jesús M. Peregrina,[†] and Søren Balling Engelsen*[,‡]

[†]Departamento de Química, Universidad de La Rioja, Centro de Investigación en Síntesis Química, U.A.-C.S.I.C., E-26006 Logroño, Spain

[‡]Quality & Technology, Department of Food Science, Faculty of Science, University of Copenhagen, Rolighedsvej 30, 1958 Frederiksberg C, Denmark

Ⓢ *Supporting Information*

ABSTRACT: In this study, La Rioja wine terroir was investigated by the use of ¹H NMR metabolomics on must and wine samples. Rioja is a small wine region in central northern Spain which can geographically be divided into three subareas (Rioja Alta, Rioja Baja, and Rioja Alavesa). The winemaking process from must, through alcoholic and malolactic fermentation, was followed by NMR metabolomics and chemometrics of nine wineries in the Rioja subareas (terroirs). Application of interval extended canonical variate analysis (iECVA) showed discriminative power between wineries which are geographically very close. Isopentanol and isobutanol compounds were found to be key biomarkers for this differentiation.

KEYWORDS: *Wine, NMR, chemometrics, metabolomics, Rioja, isopentanol, isobutanol, carbohydrates*

■ INTRODUCTION

The characterization of food authenticity and traceability is aimed at protecting the consumer and food producers from commercial or other types of fraud. In recent years, consumers have shown a renewed interest toward foods that are strongly identified with their place of origin.[1,2] In particular, traceability and authenticity of wine have been extensively investigated. Indeed, wine can be easily adulterated, due to its complex chemical composition,[3] which depends on many and diverse factors such as grape variety, environmental conditions, and enological practices. These factors have great influence on wine quality and are very important in the characterization and differentiation of wines from specific regions, such as denomination of origin.

Wine consists of several hundred components presented at different concentrations: water, ethanol, glycerol, sugars, organic acids, and some amino acids being the major ones. The main and secondary compounds are the result of the biological processes occurring such as alcoholic and malolactic fermentations. The nature and concentration of these metabolites depend on many factors, including grape types and yeasts or bacteria responsible for fermentations. Control of this process is therefore essential to obtain a quality wine.[4] In this context, the NMR emerges as a tool for monitoring and controlling several biological processes such as alcoholic and malolactic fermentations.[5]

In addition, consumers are more and more oriented toward purchasing wines with a certified authenticity and geographical origin. Authenticity testing of wine by NMR was early introduced by Martin and co-workers[6] who developed isotope NMR for detecting fraud with sugar addition and wine mixing. Currently, there is a great variety of analytical techniques employed to identify wine authenticity due to the tremendous motivation to guarantee wine quality. Most recently, metabolic profiling through metabolomics[7] have proven to be an effective method to explore and detect metabolite changes that can be used to compare, distinguish, and classify samples.[8−10] Brescia et al.[11] used NMR analysis for detecting the geographical origin of 41 red wines from various winemakers from the Apulia region (Italy). Gaudillere et al.[12−14] showed that the combination of ¹H NMR spectra with chemometric methods by multivariate statistical analysis was able to discriminate between grape samples from different environments or terroirs situated in different locations in southwestern France (Bordeaux), and in a similar study, Son et al.[15] showed that metabolomic studies could differentiate Korean grapes and their wines of different geographical origin.

In this context, the question of geographic identification of wines becomes even more interesting when it relates to small production areas or "terroir".[16] The word terroir comes from the French word *terre* and is used to describe the special characteristics of a crop related to an agricultural site including soil, weather, and farming techniques, which all contribute to the unique qualities of the crop. The need for rediscovering the true values of agriculture strictly related to terroir have led to the establishment of quality certifications labels that have become a strategic instrument of differentiation, and that gives the food products a commercial added value. Indeed, quality wines are often produced in restricted areas defined as denomination of origin (D.O.). In Rioja the qualified denomination "Denominación de Origen Calificada Rioja" (D.O.Ca. Rioja) is a small production area located in the north of Spain with 635.93 km² of vineyards divided between three subareas (Figure 1): Rioja Alta (267.86 km²), Rioja Alavesa (129.34 km²), and Rioja Baja (238.73 km²). The principal grape variety of this area is called tempranillo (*Vitis vinifera*). The whole
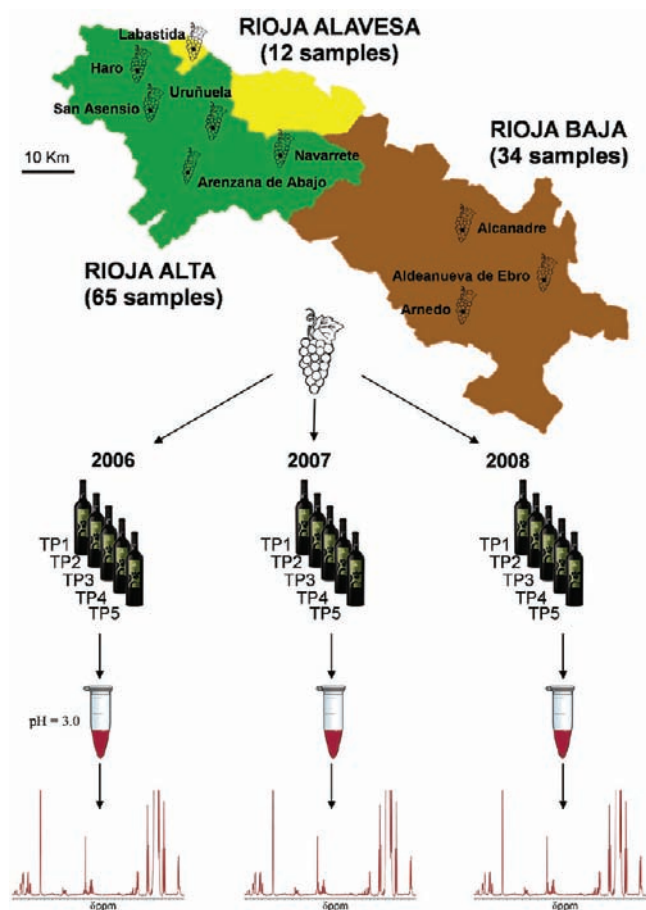
Figure 1. Overview of the geographical subareas of La Rioja region, the location of the vineyards, and a schematic summary of the experimental design.

area benefits from the confluence of two opposed climates, Atlantic and Mediterranean, which provide mild temperatures and an annual rainfall of slightly above 400 L/m^2, ideal conditions for growing grapes. For the D.O.Ca. Rioja, the rating of the harvest year (vintage ratings) is an important quality parameter and of great importance for the consumer. As a result, every year the vintages are classified into excellent, very good, good, or average in terms of quality. Recently, we have developed a quantitative method to evaluate the time course of the evolution of malic, lactic, acetic and succinic acids, proline and alanine, and ethanol in the alcoholic and malolactic fermentation of grape must from Rioja Alta wines (Bodegas Dinastia Vivanco and Bodegas Patrocinio S.C.L.).[17,18] The aim of the present work is to explore[19] the winemaking process in Rioja by ^{1}H NMR metabolomic finger-printing and advanced chemometrics[20] in order to find metabolites that are responsible for the differentiation of time points in the fermentation processes, the year of the wine production, and the origin of wine, as well as the subarea and the winery.

■ MATERIALS AND METHODS

In this study we have selected a total of nine winemaking coopera-tives, three from Rioja Baja, five from Rioja Alta, and one from Rioja Alavesa (Figure 1). The samples were collected from the vintages 2006, 2007, and 2008. During 2006, we collected five samples from each cooperative (located in Arnedo, Alcanadre, Arenzana de Abajo, Navarrete, Haro, San Asensio, Uruñuela, and Labastida) at different time points in the fermentation process: (1) before the alcoholic fermentation, (2) at the end of alcoholic fermentation, (3) at the

beginning of malolactic fermentation, (4) middle point of malolactic fermentation, and (5) after malolactic fermentation. In 2007, we collected five corresponding time point samples for each cooperative located in Arnedo, Arenzana de Abajo, Navarrete, Haro, and Uruñuela and four time point samples for those in Aldeanueva and Labastida. Finally, in 2008, we collected again five time point samples from Arnedo, Aldeanueva, Arenzana de Abajo, Navarrete, Haro, and Uruñuela, and three from Labastida. A total of 111 samples were obtained representing three vintages, nine winemaking cooperatives, and five different fermentation time points. The climatic conditions for three years are shown in Supporting Information Figure 1.

The samples were collected from the fermentation tank, transported from winery to laboratory, and preserved at −25 °C until analysis. The simplest and fastest method for recording the spectra was used, and this involved two steps. Samples were defrosted, and the pH was measured (pH Meter BasiC Crison) and adjusted to 3 by the dropwise addition of an aqueous solution of 0.1 N HCl. The must and wine samples were centrifuged at 13000 rpm for 15 min, and the supernatant (540 μL) was transferred into a 5 mm NMR tube together with D$_2$O (60 μL with the addition of the sodium salt of (trimethylsilyl) propanoic-2,2,3,3-d_4 acid (TSP) to give a final concentration of 0.58 mM in the NMR tube).

NMR Spectroscopy Analysis and Processing. NMR spectra were recorded on a Bruker Avance III 600 operating at 600.13 MHz for ^{1}H, equipped with a double tuned cryoprobe (TCI) prepared for 5 mm (o.d.) sample tubes. Acquisition of spectra was carried out with TOPSPIN software (version 3.1). Processing was performed with MestReMnova (version 6.0). The spectrometer transmitter was locked to D$_2$O frequency using a mixture H$_2$O−D$_2$O (9:1), and all the spectra were acquired at 298 K.

The ^{1}H NMR spectra were recorded with the standard pulse sequence for presaturation of the water signal at 2822.65 Hz (zgcppr program pulse). The spectral window was 20.5 ppm, and data were collected into 64k data points after 64 scans plus 2 dummy scans. The relaxation delay (d1) was set to 10 s. All NMR experiments were carried out with a fixed receiver gain (RG) which was estimate adequate through several tests. The spectra were acquired using TOPSHIM tools and the NMR SAMPLEJET that allows the automatic analysis of several samples.

Multivariate Data Analysis. Several classic and advanced chemometric tools were used for extracting relevant information from the acquired NMR data, and they are briefly described below.

Preprocessing. A normalization step was carried out on the data matrix in order to correct vertical scale errors originating from the different water content in the samples. Because no quantitative internal standard was used, all whole spectra were normalized to unit area (after having removed the spectral region containing the remaining water signal), a technique that simply equalizes the global intensity across the sample spectra not affecting the relative concentration of the peaks within each spectrum and leading to improved interpretability and quantification.[21]

Second, the normalized data matrix was overall corrected for errors in chemical shift misalignments primarily concerning pH-dependent signals using interval correlation optimized shifting (icoshift).[22]

Principal Component Analysis (PCA). PCA is a fundamental approach for exploratory (unsupervised) data analysis that displays the intrinsic data structure in a simple, low-dimensional orthogonal projection. It highlights similarities and differences among groups as well as the variables involved. The problem under investigation is usually reduced to few latent factors, i.e., principal components (PCs), sorted by significance (explained variance), which makes it easy to separate useful information from noise, while avoiding any loss of information.

In the present study, PCA was also performed separately on the three main wine ^{1}H NMR spectral regions briefly named as follow: (1) aromatic region (>5.5 ppm); (2) carbohydrate region (between 5.5 and 3.0 ppm); (3) organic acid region (<3.0 ppm). This approach accounts for different types of molecular functional groups leading to improved chemometric performance, improved simplicity, and robust-ness of the obtained models and improved interpretation. Furthermore, the three considered spectral regions have substantial differences in signal intensities that cannot be solved with a simple
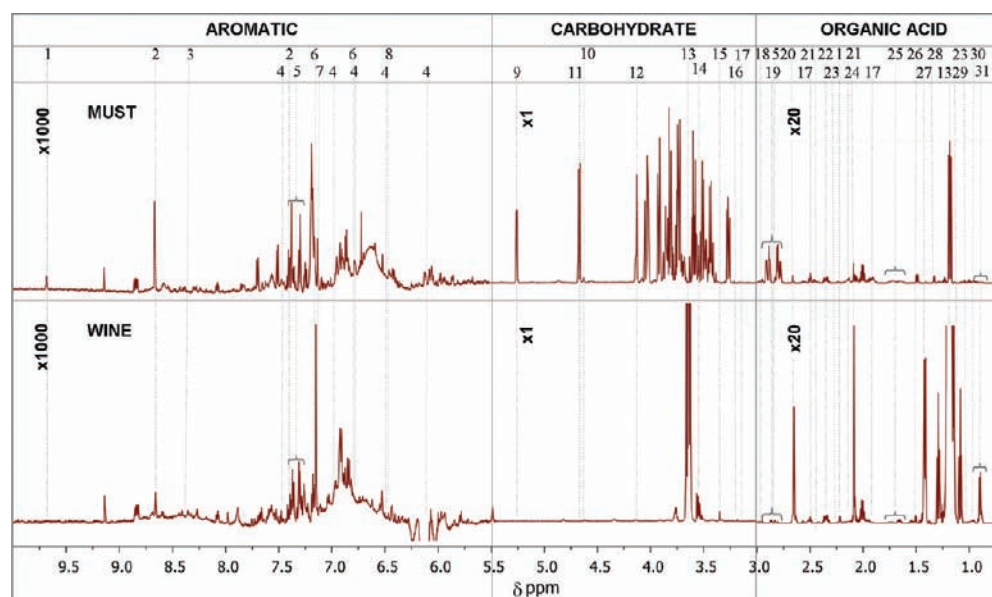
Figure 2. ^{1}H NMR spectra of must and wine including assignment of the signals listed in Table 1.

Table 1. ^{1}H NMR Chemical Shifts and Coupling Constants (Hz) of Must and Wine Compounds and Using ^{1}H NMR Spectra of Samples with Standard Added

no.	compound[a]	[1]H chemical shifts (δ) and coupling constants (Hz)	group
1	ethanal	9.66 (s), 2.22 (s)	C$\underline{H}$O, CH$_3$
2	histidine	8.66 (s), 7.39 (s)	CH, CH
3	formic acid	8.36 (s)	$\underline{H}$CO$_2$H
4	resveratrol	7.39 (d, J = 8.2), 7.01 (d, J = 16.7), 6.86−6.82 (m), 6.53 (d, J = 13.1), 6.24 (d, J = 8.1)	CH, CH, CH, CH, CH
5	2-phenylethanol	7.37 (t, aromatics), 7.29 (dd, aromatics), 2.85 (t, J = 6.8, J = 6.8)	CH, CH, CH$_2$
6	tyrosine	7.18 (d, J = 8.5), 6.88 (d, J = 8.4)	CH, CH
7	gallic acid	7.15 (s)	CH
8	fumaric acid	6.52 (s)	CH
9	α-glucose	5.23 (d, J = 7.9)	CH
10	tartaric acid	4.60 (s)	CH
11	β-glucose	4.64 (d, J = 3.7)	CH
12	α,β-fructose	4.10 (d, J = 3.7)	CH
13	ethanol	3.68 (q, J = 7.3), 1.17 (t, J = 7.3)	CH$_2$, CH$_3$
14	glycerol	3.57−3.49 (m)	CH$_2$
15	methanol	3.35 (s)	CH$_3$
16	choline	3.19 (s)	CH$_3$
17	GABA	3.13−3.09 (m), 2.49 (t, J = 7.3), 1.96 −1.87 (m)	CH$_2$, CH$_2$, CH$_2$
18	citric acid	2.96 (d, J = 15.7)	CH
19	malic acid	2.84 (dd, J = 8.0, J = 16.4)	CH$_2$
20	succinic acid	2.65 (s)	CH$_2$
21	glutamine	2.48−2.42 (m), 2.15−2.11 (m)	CH$_2$, CH$_2$
	glutamic acid	2.46−2.39 (m), 2.14−2.11 (m)	CH$_2$, CH$_2$
22	proline	2.38−2.31 (m)	CH$_2$
23	valine	2.31−2.25 (m), 1.05 (d, J = 7.0), 0.99 (d, J = 7.0)	CH, CH$_3$, CH$_3$
24	acetic acid	2.08 (s)	CH$_3$
25	arginine	1.60−1.69 (m)	CH$_2$
26	alanine	1.48 (d, J = 7.4)	CH$_3$
27	lactic acid	1.40 (d, J = 6.8)	CH$_3$
28	threonine	1.33 (d, J = 6.6)	CH$_3$
29	2,3-butanediol	1.13 (d, J = 6.3)	CH$_3$
30	leucine	0.95 (d, J = 6.8)	CH$_3$
31	isobutanol	0.87 (d, J = 6.7)	CH$_3$
	isopentanol	0.88 (d, J = 6.8)	CH$_3$
	1-propanol	0.88 (t, J = 7.5, J = 7.5)	CH$_3$

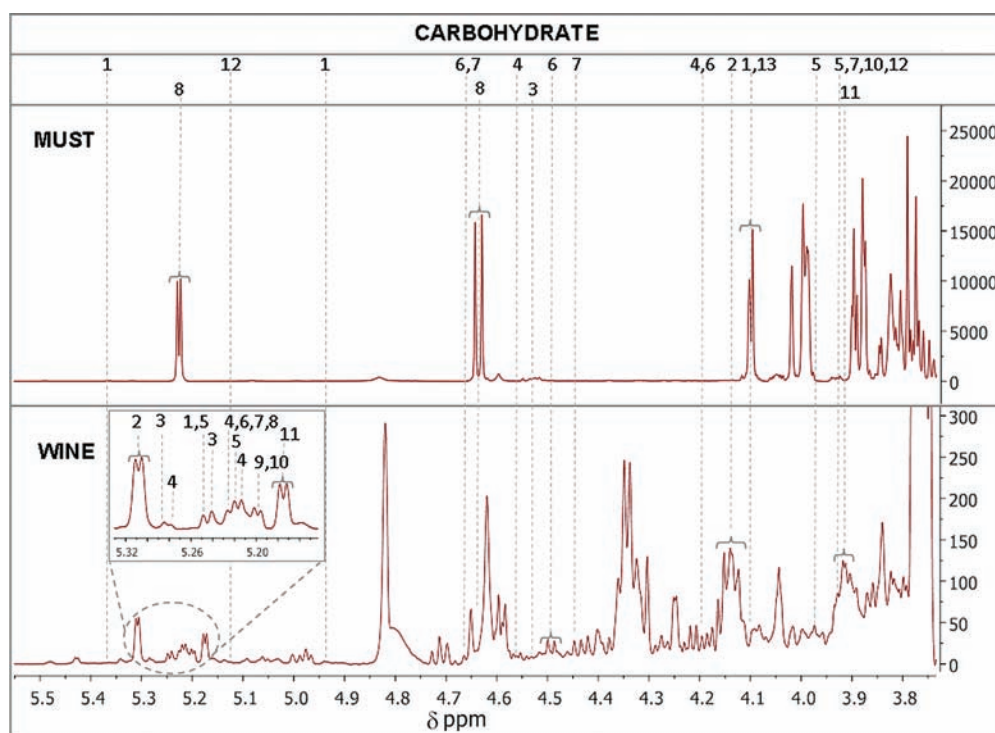[a]Identified in the literature.[9,12,18,28−33]

Figure 3. ^{1}H NMR spectra of must and wine including assignment of the carbohydrate signals listed in Table 2.

Table 2. ^{1}H NMR Chemical Shifts and Coupling Constants (Hz) of Must and Wine Carbohydrate Compounds and Using ^{1}H NMR Spectra of Samples with Standard Added

no.	compound[a]	[1]H chemical shifts (δ) and coupling constants (Hz)	carbohydrate concentration interval (mg/L)
c1	ribose	5.38 (d, $J = 3.7$), 5.25 (d, $J = 1.7$), 4.93 (d, $J = 6.5$), 4.16−4.07 (m)	3.90−28.1
c2	unknown	5.31 (d, $J = 3.7$)	
c3	arabinose	5.30 (d, $J = 4.3$), 5.24 (d, $J = 3.5$), 4.52 (d, $J = 7.8$)	5.00−144
c4	fucose	5.28 (d, $J = 4.3$), 5.23 (d, $J = 2.9$), 5.21 (d, $J = 3.8$), 4.56 (d, $J = 7.9$), 4.22−4.15 (m)	0.13−320
c5	galactose	5.25 (d, $J = 3.6$), 5.22 (d, $J = 3.2$), 3.98 (d, $J = 3.1$), 3.93 (d $J = 3.3$)	5.80−124
c6	gentibiose	5.23 (d, $J = 3.7$), 4.66 (d, $J = 7.9$), 4.50 (t, $J = 7.5$), 4.24−4.11 (m)	13.8−18.4
c7	lactose	5.23 (d, $J = 3.7$), 4.67 (d, $J = 7.9$), 4.45 (d, $J = 7.8$), 3.94 (d $J = 3.1$)	5.2−7.2
c8	glucose	5.23 (α, d, $J = 7.9$), 4.64 (β, d, $J = 3.7$)	1.64−30.1
c9	trehalose	5.20 (d, $J = 3.8$)	3.4−132
c10	xylose	5.20 (d, $J = 3.6$), 3.93 (dd, $J = 5.4$, $J = 11.5$)	0.24−58.3
c11	mannose	5.18 (d, $J = 1.2$), 3.96−3.92 (m)	2.6−194
c12	rhamnose	5.12 (d, $J = 1.4$), 3.93 (t, $J = 2.6$, $J = 2.6$)	1.14−46.0
c13	fructose	4.10 (α,β, d, $J = 3.7$)	2.9−31.6

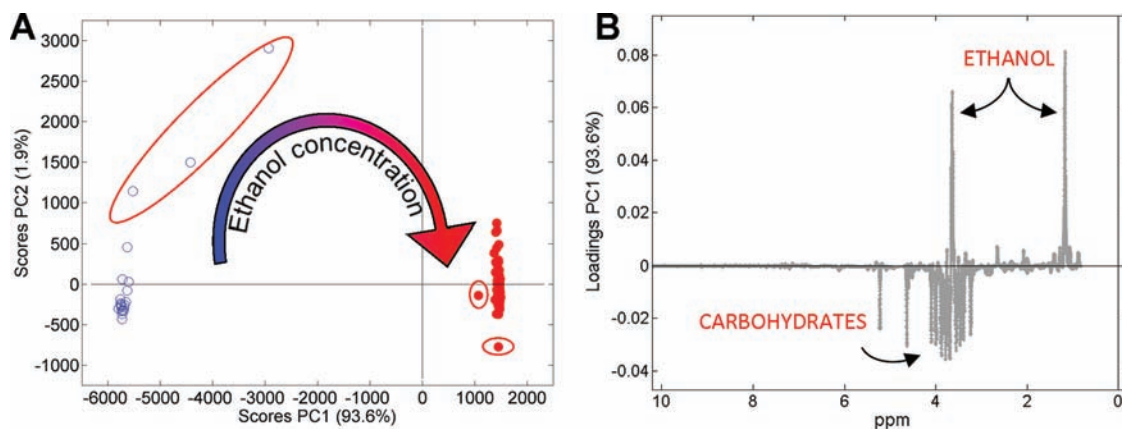[a]Identified in the literature.[34−38]

Figure 4. PCA based on the NMR spectra of the 111 samples of must and wine: (A) scores plot; (B) loading plot.
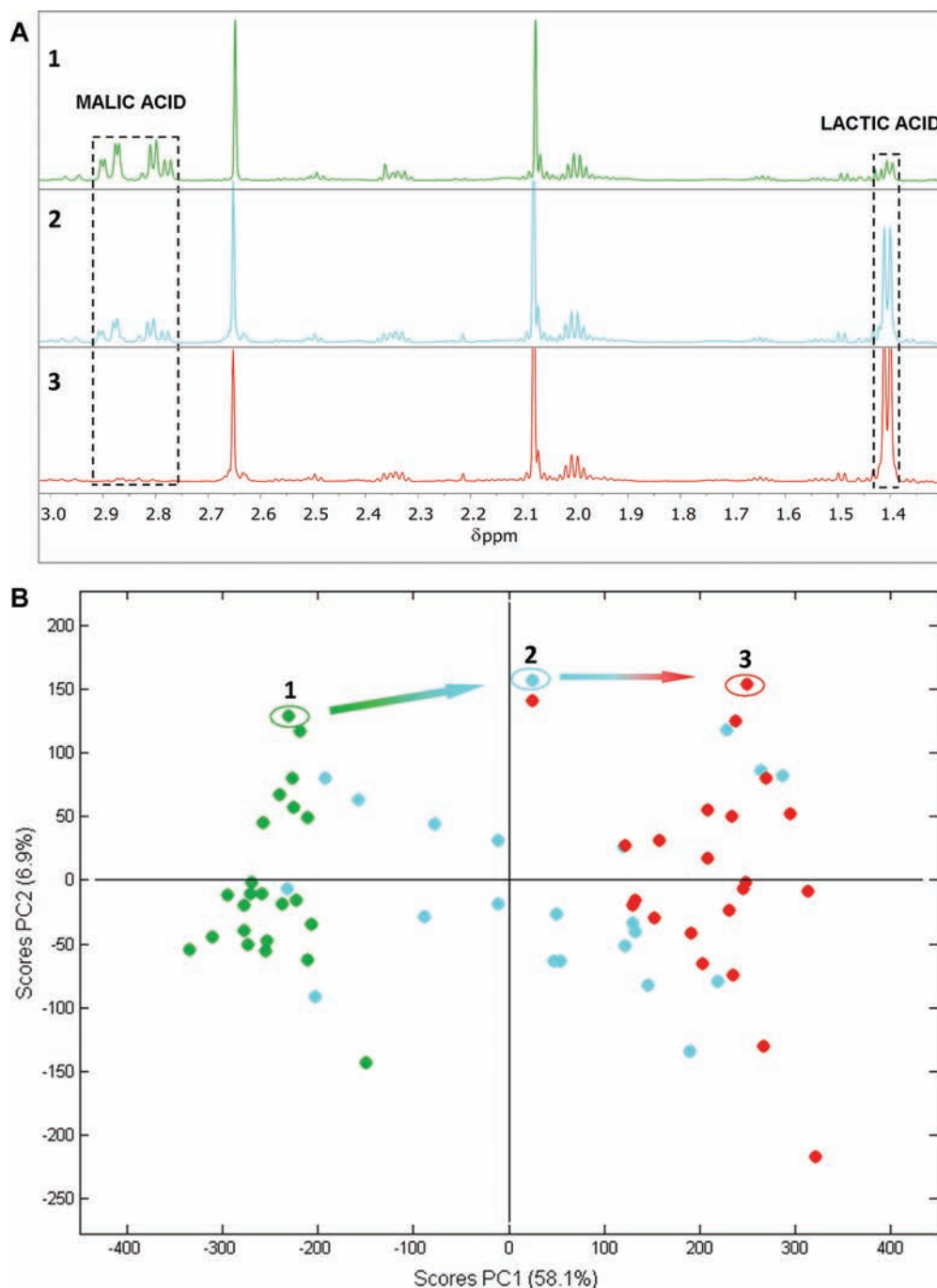
Figure 5. Overview of the changes during malolactic fermentation; (A) NMR spectra on the three different time points during malolactic fermentation; (B) PCA scores plot based on the acid region (1.3−3.2 ppm) of ^{1}H NMR spectra of must and wine (67 samples). The green points correspond to time point 1, end of alcoholic fermentation; the blue points correspond to time point 2, middle point of malolactic fermentation; the red points correspond to time point 3, end of malolactic fermentation. The time trajectory of a representative wine process is indicated by the arrows.

scaling of the data and which may lead to a loss of investigative efficacy; the approached method also compensates for this.

Extended Canonical Variable Analysis (ECVA) and Interval Extended Canonical Variable Analysis (iECVA). ECVA[23,24] is a recent chemometric classification tool representing a new approach for grouping samples based on the canonical variates analysis but extended to multivariate covariate data using an underlying PLS engine. ECVA is a powerful classification method, but because it is a supervised method careful validation is required in order to avoid overfitting. iECVA[25,26] is an extension of the iPLS concept[27] to ECVA designed to provide meaningful information about which spectral regions hold the main relevance responsible for the separation among groups. The

iECVA tool performs a series of ECV analyses, one for the whole spectrum and one for each defined interval (subregion of the spectrum having full resolution). At last, the performances of each interval are compared among each other and against the overall model, and the final results are represented in a summarizing plot that highlights the most important spectral features and enables an easier biomarker profiling. In the present application, the entire data set was subdivided into 100 segments of equal size (intervals). This segmentation does not lead to any reduction of data, like binning does, but it provides an overview of the relevant information in different spectral subdivisions.

Data alignment was performed using Matlab (2007a, The Mathworks Inc., Natick, MA) using the icoshift toolbox available at
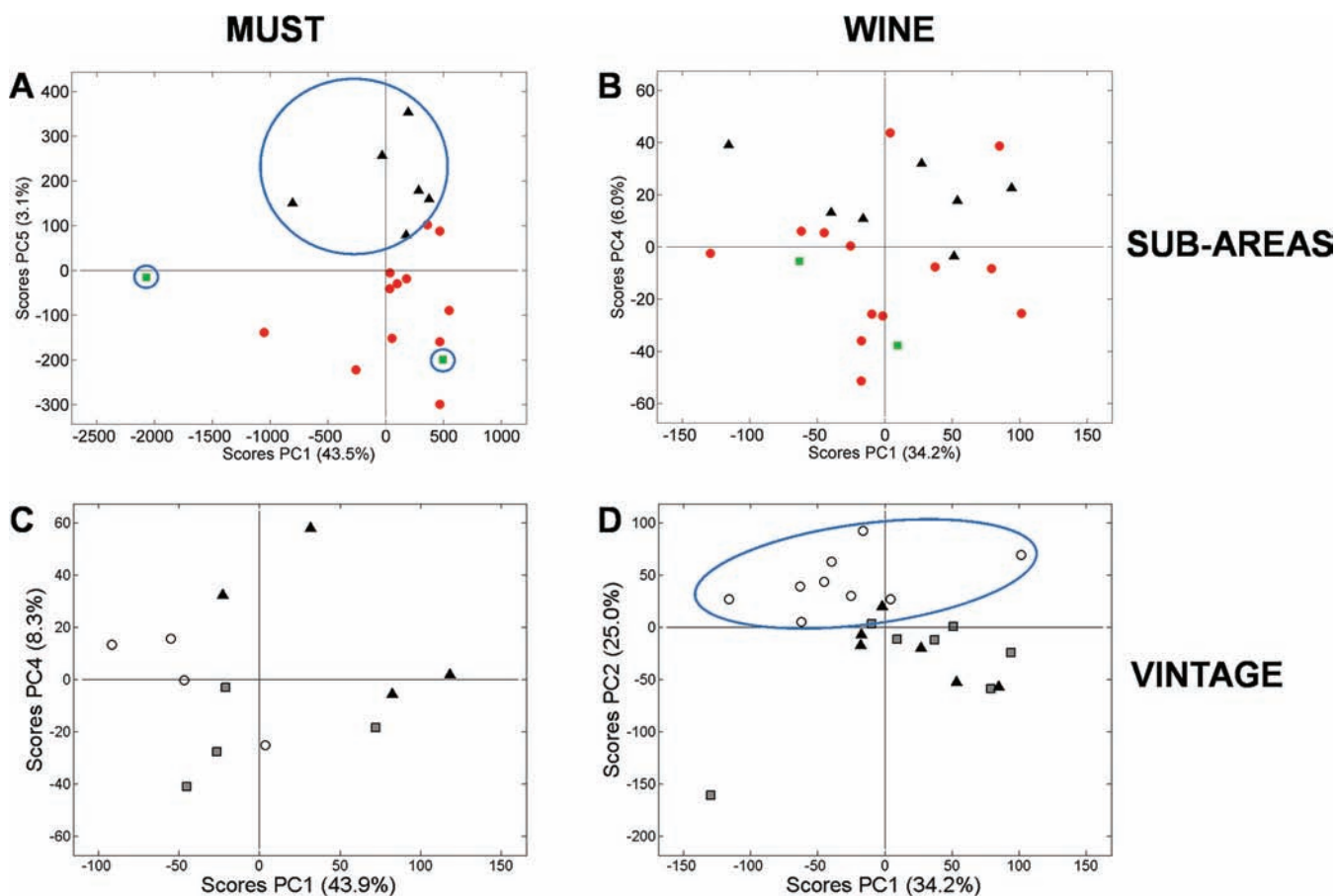
Figure 6. PCA scores plot obtained to discriminate between subareas: (A) samples of must (20 samples) using the carbohydrate region (3.2–5.5 ppm) of the spectra (a total variance of 46.6% is explained); (B) samples of wine (22 samples) using the aromatic compounds region (5.5–10.0 ppm) of the spectra (the black points correspond to samples from Rioja Baja, red points to Rioja Alta, and green points to Rioja Alavesa, and a total variance of 40.2% is explained). The PCA score plot obtained to discriminate between vintage: (C) 12 samples of must from Rioja Alta using the aromatic compound region of the spectra (a total variance of 52.1% is explained); (D) 22 samples of wine from all wineries using the aromatic compound region of the spectra (the white points correspond to samples of 2006, gray points to samples of 2007, and black points to samples of 2008, and a total variance of 59.2% is explained).

http://www.models.life.ku.dk/algorithms/. LatentiX 2.0 (www. latentix.com, Latent5, Copenhagen, Denmark), able to share data with Matlab, was used to carry out PCA analyses on the pareto-scaled data matrices. The pareto-scaling technique reduces the relative importance of large values (high intensities as for ethanol) but keeps the data structure partially intact.[26] The data were explored for information able to separate the samples according to vintage, regions, and wineries. ECVA and iECVA were carried out in Matlab using the ECVA toolbox available at http://www.models.life.ku.dk/algorithms/. Unless otherwise noted, all reported results are fully cross-validated.

■ RESULTS AND DISCUSSION

The NMR Spectra. Three different areas of the NMR spectra were analyzed separately, corresponding to organic acids (1.3–3.2 ppm), carbohydrates (3.2–5.5 ppm), aromatic compounds in must (5.5–10.0 ppm), and aromatic compounds in wine (6.4–10.0 ppm) (Figure 2). The average NMR spectra of must and wine are shown in Figure 2 including indications (1 → 31) of important assigned metabolites which are listed in Table 1. The primary difference arising in the NMR spectra moving from must to wine was the disappearance of the carbohydrate signals (sugars) in the must and the emergence of strong alcohol and organic acid signals in the wine, while the content of aromatic compounds remained relatively constant. However, in wine, the carbohydrates did not disappear completely but left a complex fingerprint, shown in Figure 3,

with the contribution of a minimum 13 different mono-saccharides or disaccharides that are assigned in Table 2.

The Alcoholic Fermentation. There are two principal processes in Rioja red wine production: alcoholic fermentation and malolactic fermentation. In the first process, sugars are transformed into ethanol and CO_2. In the second process, the most important transformation is the conversion of malic acid to the weaker lactic acid, providing a wine with a less acidic and more round taste. In order to obtain an overview of all 111 samples, a principal component analysis (PCA) was carried out. The resulting scores and loadings plot, Figure 4, show a complete differentiation between must and wine samples (when alcoholic fermentation is completed). The major variation in the NMR data is thus the ethanol content, and indeed four samples of must show a deviating pattern because they were collected at a time when the alcoholic fermentation had already begun.

The Malolactic Fermentation. In order to investigate the malolactic fermentation, a PCA was conducted including only the 67 wine samples that are distributed according to the three malolactic fermentation time points: (1) end of alcoholic fermentation; (2) middle point of malolactic fermentation; (3) end of malolactic fermentation. In this case, only the organic acid area from spectra was taken into consideration (Figure 5A shows spectra for three samples that belong to each time point during the malolactic fermentation). The main metabolic change is the transformation
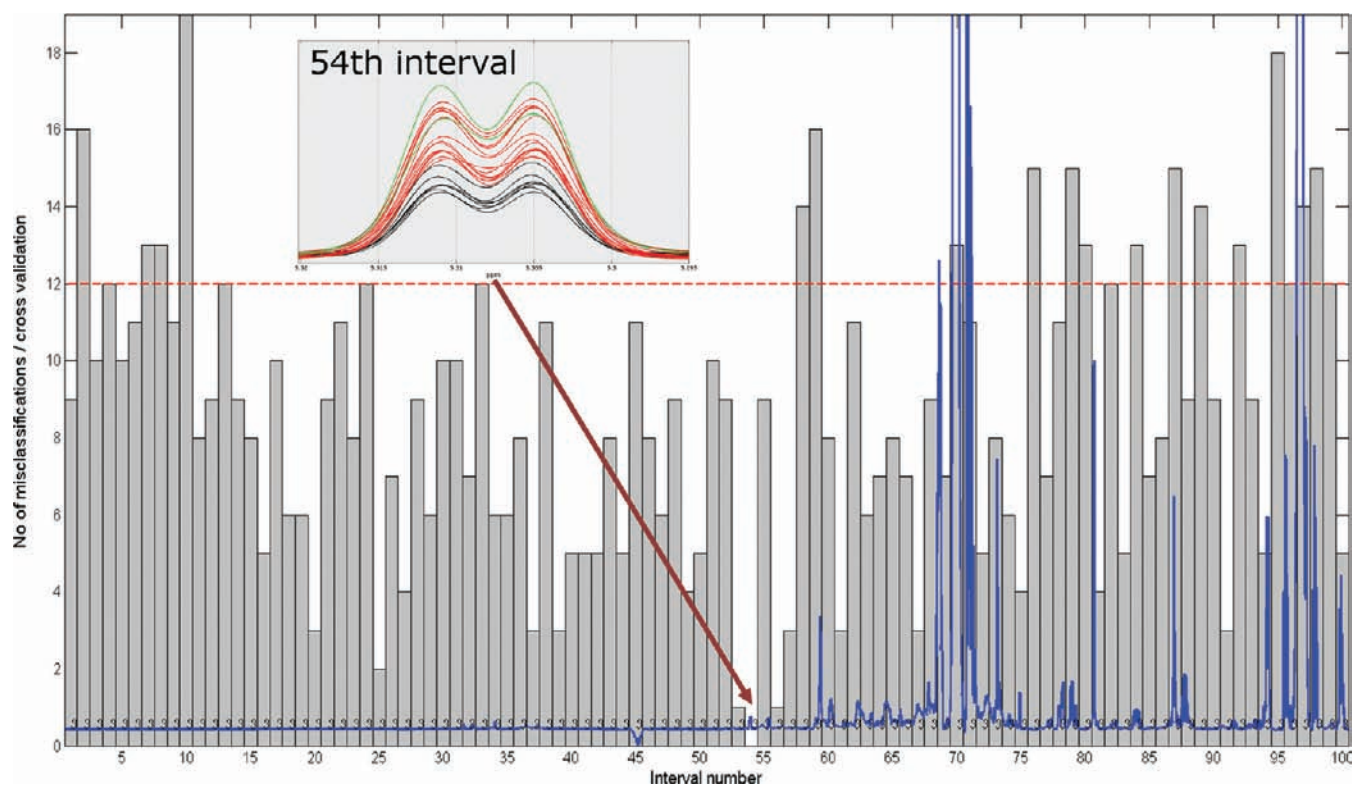
Figure 7. iECVA plot for the classification of the two major subareas in la Rioja based on 26 wine samples of the 2007 vintage. The plot shows the number of misclassifications (bars) for each spectral interval and for the global spectral model (red stipulated line). The NMR spectra are an average of the 26 sample spectra.
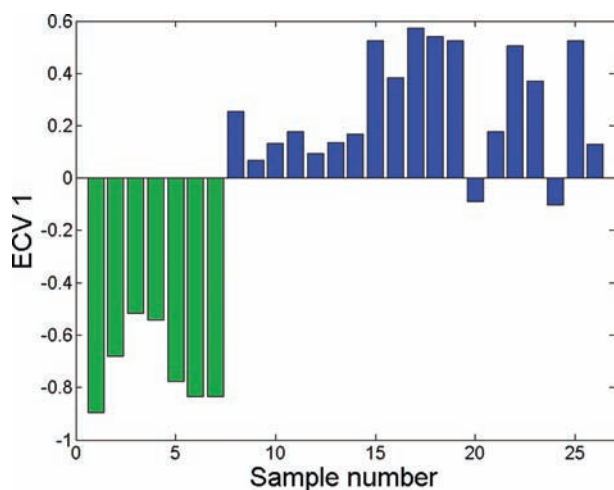

Figure 8. ECVA score plot from the 54th interval in the 2007 vintage. Rioja Alta and Rioja Alavesa are shown in blue, and Rioja Baja is plotted in green.

of malic acid (2.84 ppm) into lactic acid (1.40 ppm). Control of this fermentation process is essential in order to obtain a balanced wine of high quality. The PCA scores plot (Figure 5B) shows the three malolactic fermentation time points.

Differentiation of the Subareas inside Rioja D.O.Ca. Discrimination of the three subareas of Rioja was investigated for both must and wine samples. Figure 6A shows a PCA score plot of the 20 must samples based on the carbohydrate region of the spectra which was the only discriminative region. The score plot in Figure 6A shows that two subareas are clearly separated: Rioja Alta plus Rioja Alavesa and Rioja Baja. Only

the winery in Rioja Alavesa (located in Labastida) cannot be distinguished, presumably because it is a very close neighbor to the subarea of Rioja Alta (Figure 1). In case of the final wine samples, the best discriminative spectral region proved to be the aromatic one. The PCA scores plot derived from this region is shown in figure 6B and reveals that a complete classification on the subareas cannot be obtained using this unsupervised analysis even though a clear tendency in this direction can be observed. Taking data from 22 final wine samples and using the spectrum in the area of aromatic compounds, plot of the scores in PC4 versus PC1 space explains 40.2% of the total variance (Figure 6B).

The Vintage. Discrimination based on the production year was also investigated using PCA. Using 12 samples of must from Rioja Alta, and the aromatic compound region of the NMR spectra (5.5–10.0 ppm), the scatter plot of PC4 versus PC1 scores is shown in Figure 6C, showing three different groups corresponding to vintages 2006, 2007, and 2008 but providing only weak indication for a discrimination. In contrast, the vintage of 2006 was clearly distinguished in a PCA scores plot, obtained from the aromatic spectral region (6.4–10.0 ppm), of the 22 final wine samples (Figure 6D). This result has been supported by the average temperature measured in the different areas of la Rioja (see Figure 1 in Supporting Information).

Interval Extended Canonical Variate Analysis (iECVA). In order to scrutinize the spectra for signals able to distinguish geographical regions, interval extended canonical variate analysis (iECVA) was carried out on the wine spectra using 100 equally sized subintervals. Using this procedure, and assuming that the samples originating from Rioja Alta and Alavesa belong to the same group (as suggested by the PCA scores plot in Figure 6A and 6B), one interval was found able to significantly improve the classification (Figure 7).
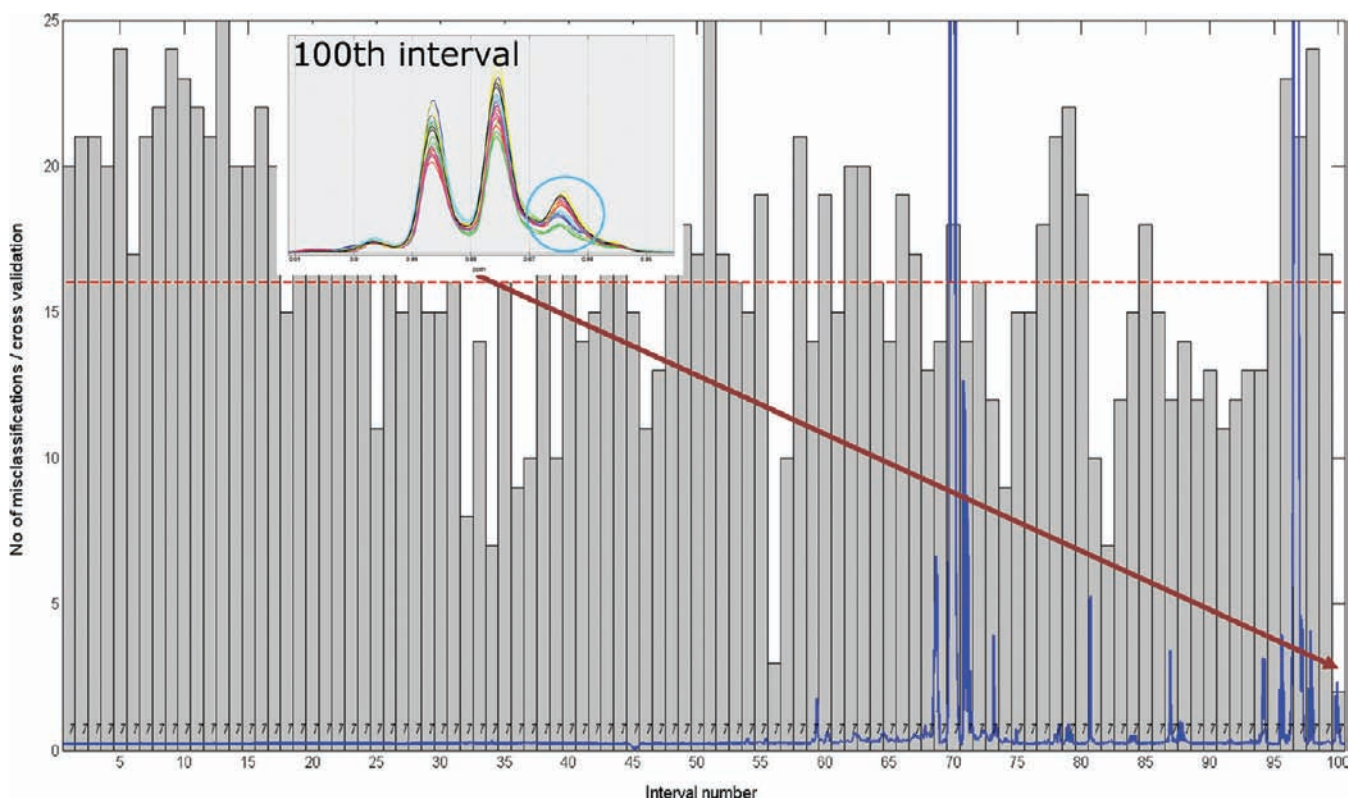
Figure 9. iECVA plot ¹H NMR average spectra of 26 wine samples of the 2007 vintage indicating the best interval (no. 100) for the lowest number of misclassifications in order to evaluate different wineries. The highlighted interval is shown in the zoomed inset containing the signals arising from isobutanol and isopentanol substances.
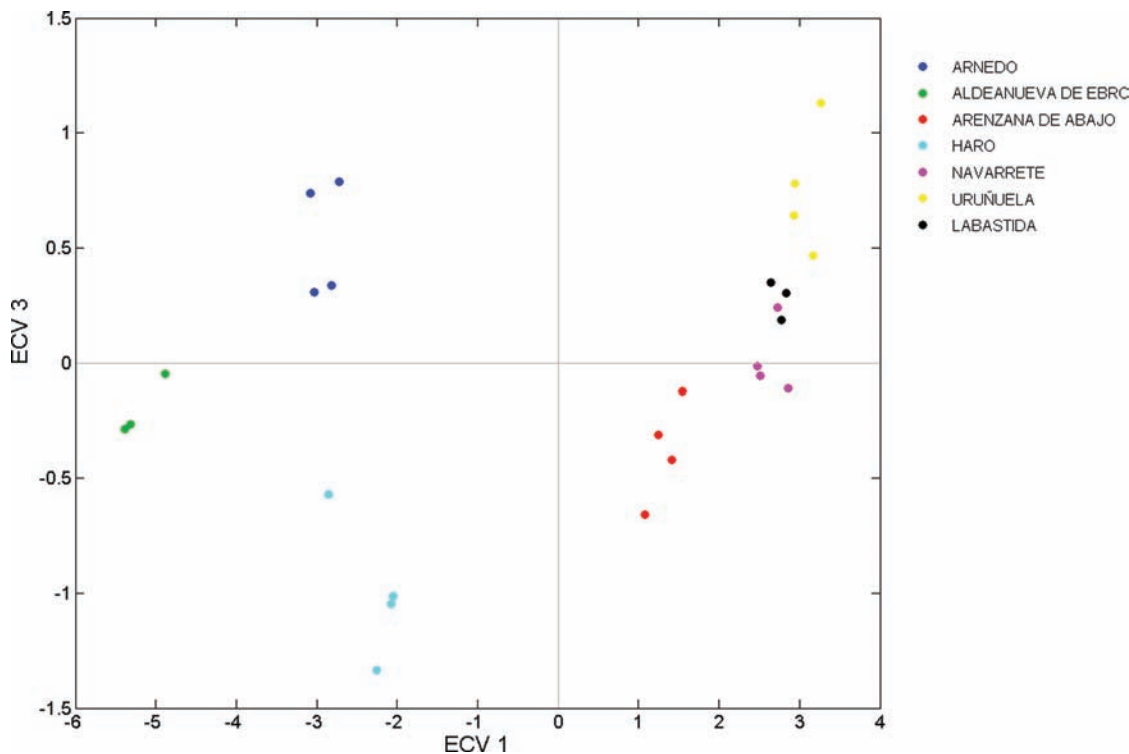

Figure 10. ECVA score plot from the 100th interval (0.83−0.93 ppm) in the 2007 vintage showing the discrimination between individual wineries in la Rioja.

The iECVA plot in Figure 7 reveals that the 54th interval (5.25−5.35 ppm) was able to reduce the number of misclassifications from 5 to 0 with respect to the global model. This interval includes unidentified signals of some anomeric protons from a carbohydrate molecule (unknown compound in Table 2), and in literature it has been assigned to

an anthocyanin linked to a glyceryl carrier.[28] In Rioja wines studied in this work, this substance or class of substances is able to perfectly discriminate subareas in the three studied years. In Figure 8, we show the bar charts for 2007 (bar charts of 2006 and 2008 vintages are reported in Supporting Information Figures 2 and 3). Using the 26 samples belonging to the 2007 vintage, only three components were sufficient for obtaining the complete differentiation with 0 misclassifications (0.00% error).

iECVA was also applied in an attempt to investigate signals able to distinguish the single wineries. Indeed, an interesting interval was found by iECVA, which was able to improve the classification rate significantly (Figure 9). The 100th interval (0.83−0.93 ppm) was able to reduce the number of misclassifications from 16 to 2 with respect to the global model. This interval includes two signals corresponding to isobutanol (0.87 ppm, d, ^3J = 6.71 Hz) and isopentanol (0.88 ppm, d, ^3J = 6.76 Hz). The signals were confirmed by spiking, adding the pure compounds to the wine samples and acquiring new NMR spectra.

Apparently, isobutanol and isopentanol contain effective information about the differentiation between wineries across the three vintages. Figure 10 shows the iECVA score plots for 2007 (score plots for 2006 and 2008 are reported in Supporting Information Figures 4 and 5). Using the 26 samples belonging to the 2007 vintage, seven components were required in order to obtain the differentiation with two misclassifications (7.69% error). It should be emphasized that with this small interval, it is possible to differentiate wines from wineries that are in close geographical proximity. Isopentanol and isobutanol may be considered as significant biomarkers for the differentiation of individual wineries from this wine region.

In conclusion, we have demonstrated that the winemaking process in la Rioja can be efficiently explored by means of ^{1}H NMR metabolite profiling of wine and must. The study shows that the musts and wines can be differentiated in time points of the fermentation processes, in subareas, and also to a certain extent in different vintages. Moreover, by means of extended cannonical variates analysis of ^{1}H NMR spectral intervals, a very good discrimination was found even at the individual winery level. This latter finding is remarkable because the wineries are in close geographical proximity and because a small NMR spectral region, which is assigned to resonances from isopentanol and isobutanol, was found to contain the information for such a discrimination, revealing these substances to be important biomarkers of la Rioja terroir.

ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Fax: +34 941 299621. E-mail: hector.busto@unirioja.es (J.H.B); se@life.ku.dk (S.B.E.).

Author Contributions

§These authors contributed equally to the research.

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